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Identification of an Emerging Pathogen, *Mycobacterium massiliense*, by *rpoB* Sequencing of Clinical Isolates Collected in the United States▽

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***Mycobacterium massiliense* is a rapidly growing mycobacterium that is indistinguishable from *Mycobacterium chelonae*/*M. abscessus* by partial 16S rRNA gene sequencing. We sequenced *rpoB*, *sodA*, and *hsp65* genes from isolates previously identified as being *M. chelonae*/*M. abscessus* and identified *M. massiliense* from isolates from two patients with invasive disease representing the first reported cases in the United States.**

Rapidly growing mycobacterium infections are increasing in the United States (7) and are difficult to speciate by conventional methods. Partial 16S rRNA gene sequencing is the most widely used method for the identification of nontuberculous mycobacteria (6, 8, 12), but this gene target is often limited by the lack of sequence divergence among closely related *Mycobacterium* species (15). *Mycobacterium chelonae* and *M. abscessus* are two species that share the same 16S rRNA gene sequence, and since distinguishing these two species is clinically relevant, assays targeting base pair differences within the 16S-23S rRNA internal transcribed spacer (ITS) region have been developed (5). The ITS assay has proven to be valuable but cannot differentiate *M. abscessus* from *M. massiliense* and *M. bolletii*, two new species of mycobacteria that share the same 16S rRNA gene sequence with *M. chelonae*/*M. abscessus* (1, 4). Although *M. massiliense* and *M. bolletii* have not been described in the United States, these two species may have been misclassified by previous assays and may remain undetected as emerging pathogens.

We sequenced portions of the *rpoB*, *sodA*, and *hsp65* genes to gain a better understanding of the frequency of detection of *M. massiliense* or *M. bolletii* among clinical isolates identified as being *M. chelonae*/*M. abscessus* by 16S and ITS assays. From this analysis, we found four isolates from two patients with identifications consistent with the novel species *M. massiliense* and report their clinical case histories. To our knowledge, these are the first reported cases of invasive infections from *M. massiliense* in the United States.

TABLE 1. Comparison of gene sequences from clinical isolates and reference strains of *M. abscessus*, *M. chelonae*, and *M. massiliense*

Organism	No. of isolates found by ITS assay	*rpoB*[a]		*sodA*[b]		*hsp65*[c]	
		No. of isolates	% Identity	No. of isolates	% Identity	No. of isolates	% Identity
M. abscessus	55	47	100	42	100	44	100
		2	99.9	2	99.8		
		1	99.6				
		1	99.3				
M. chelonae	8	1	100	4	100	6	100
		4	99.7	2	99.8	1	99.8
		3	99.6	1	99.5	1	98.8
				1	98.4		
M. massiliense	NA[d]	4	99.3	11	100	11	100

[a] *rpoB* reference sequences under GenBank accession numbers AY147164 for *M. abscessus*, AY147163 for *M. chelonae*, and AY593981 *M. massiliense*.
[b] *sodA* reference sequences under GenBank accession numbers AY458102 for *M. abscessus*, AY458104 for *M. chelonae*, and AY593975 for *M. massiliense*.
[c] *hsp65* reference sequences under GenBank accession numbers AY498743 for *M. abscessus*, AY458082 for *M. chelonae*, and AY596465 for *M. massiliense*.
[d] NA, not applicable.

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Case reports. (i) Patient 1. A 43-year-old female from Nevada with multiple sclerosis and pacemaker placement 11 months previously presented with pacemaker pocket infection. An erythematous "lump" developed at her pacemaker site that required local incision and drainage (no culture). She was treated empirically with vancomycin but developed fever and increasing pain at the site. Intraoperative cultures from surgical debridement grew colonies of acid-fast bacilli, which were identified as being *M. abscessus*. She remained on vancomycin. The fever persisted, and 2 weeks later, all components of the pacemaker were removed surgically, with intraoperative cultures again being positive for *M. abscessus*. The isolate was susceptible only to clarithromycin (MIC < 0.12 μg/ml) and amikacin (MIC < 16 μg/ml). She was discharged and received 6 months of clarithromycin treatment.

(ii) Patient 2. A 29-year-old female from Florida with chronic myelogenous leukemia received an allogeneic hematopoietic stem cell transplant that was complicated by chronic graft-versus-host disease. Five months following the hematopoietic stem cell transplant, she developed fever and cough. Cultures from bronchoalveolar lavage and multiple blood cultures recovered rapidly growing mycobacteria identified as being *M. abscessus*. The isolate was susceptible to linezolid (MIC < 8 mg/ml), clarithromycin (MIC < 0.12 μg/ml), and amikacin (MIC < 16 μg/ml). She received 6 weeks of treatment with intravenous tigecycline, oral moxifloxacin, and oral azithromycin.

Over a 7-month period, 63 clinical isolates, representing 58 patients, that were previously identified as harboring *M. chelonae* ($n = 8$) and *M. abscessus* ($n = 55$) by 16S and ITS sequence analyses (5) were retrieved retrospectively. DNA extractions, PCR, and sequencing reactions were performed as previously described (13) with amplification and sequencing primers targeting the *rpoB* (2), *sodA* (3), and *hsp65* (14) genes. Neighbor-joining trees were constructed by using MEGA v3.1 (9). Isolates were identified by using the *rpoB* sequence criteria described previously by Adekambi et al. (2). Susceptibility testing was performed by broth microdilution according to CLSI (formerly NCCLS) standard M24-A (11). Doxycycline susceptibility testing was performed by Etest (AB Biodisk, Solna, Sweden).

A comparison of *rpoB*, *sodA*, and *hsp65* sequences is shown in Table 1. Four isolates identified as being *M. abscessus* by ITS assay were identified as being *M. massiliense* by *rpoB* sequencing (Fig. 1). The *rpoB* sequencing results agreed with results for the ITS assay for the remaining 59 isolates. With *sodA* sequencing, only *M. chelonae* was differentiated from *M. massiliense* and *M. abscessus* (Figure). A total of 11 isolates had 100% identity with the previously published *sodA* sequence for *M. massiliense.* However, the *rpoB* sequence identified these 11 isolates as being *M. massiliense* ($n = 4$) or *M. abscessus* ($n = 7$). A comparison of the *hsp65* sequences showed results that were similar to those of *sodA* comparisons. The 11 isolates that shared *hsp65* identity with *M. massiliense* correlated with the 11 isolates that shared 100% *sodA* identity with *M. massiliense.* No isolates were identified as being *M. bolletii*. Susceptibility patterns for all 63 isolates are shown in Table 2. All *M. massiliense* isolates were resistant to doxycycline.

With *rpoB* sequencing, 51 isolates originally identified as *M. abscessus* isolates by ITS assay were confirmed to be *M. abscessus* isolates, with 4 isolates recharacterized as harboring *M. massiliense*. To our knowledge, this is the first report to describe the isolation and identification of *M. massiliense* isolates collected in the United States associated with invasive infections, and it suggests that *M. massiliense* may be more com-

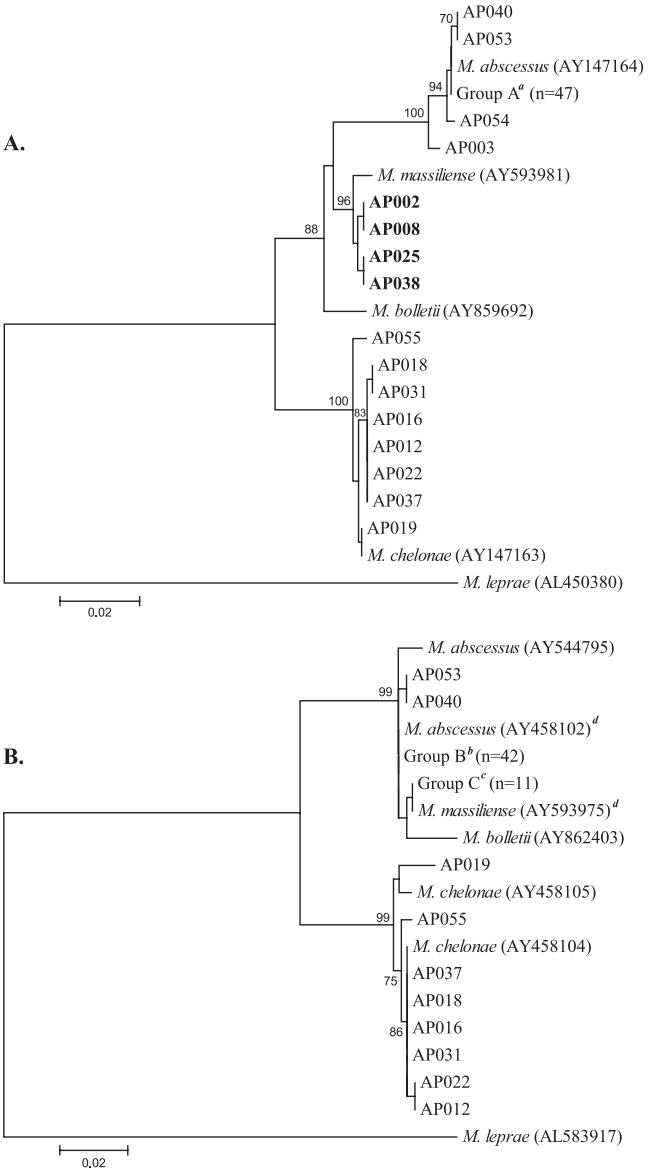

FIG. 1. Phylogenetic trees of *rpoB* (A) and *sodA* (B) gene sequences. Bootstrapping values over 70% are recorded at the tree nodes. *Mycobacterium leprae* was used as an outgroup. Study isolates are designated AP001 to AP063. Isolates identified as being *M. massiliense* by *rpoB* sequencing are in boldface type. Isolates AP002 and AP008 represent patient 1, and isolates AP025 and AP038 represent patient 2. [a]Includes isolates AP001, AP004 to AP007, AP009 to AP011, AP013 to AP015, AP017, AP020, AP021, AP023, AP024, AP026 to AP030, AP032 to AP036, AP039, AP041 to AP052, and AP056 to AP063. [b]Includes isolates AP004 to AP007, AP009 to AP011, AP013 to AP015, AP017, AP020, AP021, AP023, AP024, AP026 to AP028, AP030, AP032 to AP036, AP039, AP041, AP042, AP044 to AP052, AP056, AP059, and AP061 to AP063. [c]Includes isolates AP001, AP002, AP003, AP008, AP025, AP029, AP038, AP043, AP057, AP058, and AP060. [d]Published *sodA* sequences for *M. massiliense* (GenBank accession number AY593975) and *M. abscessus* (accession number AY458102) differed by only 2 bp.

TABLE 2. Antimicrobial susceptibilities of isolates by broth microdilution method

Identification based on *rpoB* sequence (no. of isolates)	MIC (μg/ml) (no. of isolates)									
	Linezolid	Clarithromycin	Amikacin	Cefoxitin	Imipenem	Tobramycin	Ciprofloxacin	Gatifloxacin	Minocycline	Trimethoprim-sulfamethoxazole
M. abscessus[a] (51)	2 (1) 4 (4) 8 (15) 16 (22) 32 (7)	0.12 (20) 0.25 (8) 0.5 (8) 1 (10) 2 (5)	8 (12) 16 (23) 32 (14)	16 (9) 32 (31) 64 (9)	1 (2) 4 (7) 8 (27) 16 (10) 32 (3)	4 (2) 8 (12) 16 (30) 32 (5)	0.25 (1) 4 (6) 8 (8) 16 (20) 32 (14)	0.5 (1) 4 (9) 8 (10) 16 (29)	16 (2) 64 (47)	4/76 (2) 8/152 (13) 16/304 (34)
M. massiliense (4)	32 (4)	0.12 (4)	16 (4)	32 (4)	8 (4)	16 (3) 32 (1)	16 (3) 32 (1)	16 (4)	8 (2) 64 (2)	8/152 (2) 16/304 (2)
M. chelonae (8)	2 (1) 4 (4) 8 (3)	0.12 (5) 0.25 (3)	8 (1) 16 (3) 32 (4)	256 (1) 512 (7)	4 (1) 8 (3) 16 (4)	1 (4) 2 (4)	0.5 (1) 1 (1) 2 (2) 4 (2) 8 (1) 16 (1)	1 (1) 2 (3) 4 (2) 8 (2)	32 (3) 64 (5)	4/76 (2) 8/152 (4) 16/304 (2)

[a] Two *M. abscessus* isolates had susceptibility testing performed for clarithromycin only.

monly encountered but may be potentially misclassified as *M. abscessus*.

Unlike partial 16S rRNA gene sequencing, where interspecies similarity is high, with ≤0.4% sequence differences, the *rpoB* gene sequence is more variable, with 2 to 3% sequence differences correctly classifying most nontuberculous mycobacteria (9). In this study, we found partial *rpoB* gene sequencing to be a more discriminating gene target than *sodA* and *hsp65* for *M. massiliense*, a unique observation that conflicts with data from previous reports (4, 10). Additionally, other investigators previously proposed that susceptibility to doxycycline may serve as a surrogate marker to differentiate *M. abscessus* from *M. massiliense* (4), but we could not confirm this finding.

Distinguishing *M. chelonae* from *M. abscessus* is clinically important because of their unique susceptibility patterns and disease manifestations. Our understanding of *M. massiliense* representing a distinct clinical and taxonomical entity from *M. abscessus* is still evolving. For our two patients, no histories of unusual exposure to animals or environmental sources were present, and their clinical presentations and susceptibility patterns were similar to those of infections caused by *M. abscessus*.

Overall, *rpoB* gene sequencing is emerging as the preferred tool to identify mycobacteria taxa (1–4) and enabled us to identify *M. massiliense* from two patients with invasive infection. Although the clinical significance of routinely identifying certain mycobacteria remains unclear, the 16S rRNA gene and ITS regions are often inadequate to completely capture the microbial diversity of mycobacteria, and alternative gene targets such as the *rpoB* gene should be considered to recognize emerging mycobacterial pathogens with the potential for invasive disease.

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REFERENCES

1. **Adekambi, T., P. Berger, D. Raoult, and M. Drancourt.** 2006. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. Int. J. Syst. Evol. Microbiol. **56:**133–143.
2. **Adekambi, T., P. Colson, and M. Drancourt.** 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41:**5699–5708.
3. **Adekambi, T., and M. Drancourt.** 2004. Dissection of phylogenetic relationships among 19 rapidly growing *Mycobacterium* species by 16S rRNA, *hsp65*, *sodA*, *recA* and *rpoB* gene sequencing. Int. J. Syst. Evol. Microbiol. **54:**2095–2105.
4. **Adékambi, T., M. Reynaud-Gaubert, G. Greub, M.-J. Gevaudan, B. La Scola, D. Raoult, and M. Drancourt.** 2004. Amoebal coculture of "*Mycobacterium massiliense*" sp. nov. from the sputum of a patient with hemoptoic pneumonia. J. Clin. Microbiol. **42:**5493–5501.
5. **Cloud, J. L., K. Hoggan, E. Belousov, S. Cohen, B. Brown-Elliot, L. Mann, R. Wilson, W. Aldous, R. J. Wallace, and G. L. Woods.** 2005. Use of the MGB Eclipse system and SmartCycler PCR for differentiation of *Mycobacterium chelonae* and *M. abscessus*. J. Clin. Microbiol. **43:**4205–4207.
6. **Cloud, J. L., H. Neal, R. Rosenberry, C. Y. Turenne, M. Jama, D. R. Hillyard, and K. C. Carroll.** 2002. Identification of *Mycobacterium* spp. by using a commercial 16S ribosomal DNA sequencing kit and additional sequencing libraries. J. Clin. Microbiol. **40:**400–406.
7. **De Groote, M. A., and G. Huitt.** 2006. Infections due to rapidly growing mycobacteria. Clin. Infect. Dis. **42:**1756–1763.
8. **Hall, L., K. A. Doerr, S. L. Wohlfiel, and G. D. Roberts.** 2003. Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. J. Clin. Microbiol. **41:**1447–1453.
9. **Kumar, S., K. Tamura, and M. Nei.** 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. **5:**150–163.
10. **McNabb, A., K. Adie, M. Rodrigues, W. A. Black, and J. Isaac-Renton.** 2006. Direct identification of mycobacteria in primary liquid detection media by partial sequencing of the 65-kilodalton heat shock protein gene. J. Clin. Microbiol. **44:**60–66.
11. **NCCLS.** 2003. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes: approved standard. NCCLS document M24-A. NCCLS, Wayne, PA.
12. **Patel, J. B., D. G. B. Leonard, X. Pan, J. M. Musser, R. E. Berman, and I. Nachamkin.** 2000. Sequence-based identification of *Mycobacterium* species using the MicroSeq 500 16S rDNA bacterial identification system. J. Clin. Microbiol. **38:**246–251.
13. **Simmon, K. E., A. C. Croft, and C. A. Petti.** 2006. Application of SmartGene IDNS software for partial 16S rRNA gene sequences for a diverse group of bacteria in a clinical laboratory. J. Clin. Microbiol. **44:**4400–4406.
14. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Bottger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
15. **Tortoli, E.** 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin. Microbiol. Rev. **16:**319–354.